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Effect of side chain structure on aggregation state and mechanical properties of synthetic polypeptide monolayers at the air-water interface

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Synopsis

Aggregation structure of poly(γ-methyl-L-glutamate)(PMLG) and poly(γ-n-hexyl-L-glutamate)(PnHLG) monolayers at the air-water interface was investigated on the basis of the transmission electron microscopic observation. The bright field image of PMLG and PnHLG monolayers exhibited a homogeneous structure at surface pressure of 18 $mN \cdot m^{-1}$ and 15 $mN \cdot m^{-1}$, respectively. The bright field image of PMLG monolayers showed linear humps at high surface pressure, suggesting the collapse of monolayers and the orientation of α-helix axis perpendicular to the compression direction. Surface pressure anisotropy was investigated by means of orthogonal Wilhelmy plate method. The surface pressure anisotropy for PMLG was greater than that for PnHLG. Dynamic viscoelastic properties of polypeptides monolayers at the air-water interface were examined on the basis of transient response of surface pressure. The magnitude of dynamic storage modulus E' of the PnHLG monolayer was lower than that of PMLG monolayer. This is ascribed to the active side chain motion of PnHLG at room temperature. The mechanical properties and aggregation structure of polypeptide monolayers were revealed to depend on their thermal molecular motion of side chain.

Introduction

Recently, Langmuir-Blodgett (LB) films (1) have been paid great attention due to its potential application for the biomedical, optical, and electronic fields (2). LB films were obtained by transferring monolayers spread at the air-water interface to a solid substrate. These films have several problems such as low mechanical strength and thermal instability. These defects may be attributed to weak interaction among molecules in a monolayer. Preparation of LB films utilizing polymeric molecules are one of the methods to improve mechanical strength and thermal stability.

In order to prepare large-area and defect-free LB films, it is necessary to understand mechanical properties of monolayers at the air-water interface. Canal viscometers (3,4), surface pressure relaxation (5), torsion pendulums (6-7), longitudinal waves (8-10) and surface light scattering (SLS) (11-12) methods have been used to examine the mechanical properties of fatty acid monolayers at the air-water interface. However, little investigation has been done on the mechanical properties of polymeric monolayers.

In this study, polypeptides with different side chain group were used as polymeric monolayers. Polymeric LB films of polypeptide have been studied because polypeptide monolayers are important as simple model of protein in biological membranes. The effect of side chain structure on morphology and surface pressure anisotropy of polypeptide monolayers was investigated based on a transmission electron microscopy. Also, dynamic viscoelastic properties of polypeptide monolayers at the air-water interface were investigated on the basis of transient response of surface pressure after application of step rise compression strain.

Experimental

Materials

Poly(γ-methyl-L-glutamate)(PMLG) and poly(γ-n-hexyl-L-glutamate)(PnHLG) have been used for a preparation of monolayers. These polypeptides were different in their side chain length. PMLG was supplied by Ajinomoto Co., Ltd. The viscosity-average molecular weight was 140,000.

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PnHLG was prepared by the alcoholysis method using PMLG as the starting materials (13). It is widely accepted that three different secondary structures, the α-helix, the β-pleated sheet, and the random coil, exist in the solid or solution states of synthetic polypeptides, depending on the hydrogen bonding capability of solvents (14) used for preparation.

Monolayer Preparation

PMLG and PnHLG were dissolved in chloroform ($CHCl_3$) at a concentration of about 0.05 wt %. Chloroform is a α-helix-forming solvent. Their chloroform solutions were spread at the air-water interface, resulting in the formation of monolayers. Highly purified water by Milli-Q II ^{TM} system (Millipore Co., Ltd.) was used as a subphase. Surface pressure (π)-area (A) isotherms were measured by compressing the spread monolayers at a constant speed of 0.16 $cm.min^{-1}$. Surface pressure was monitored by Wilhelmy balance with a microprocessor-controlled barrier (FSD, San-esu Keisoku Co., Ltd., Fukuoka, Japan).

Morphological Observation of Monolayer

The aggregation structure of monolayers at the air-water interface was investigated by a transmission electron microscope (Hitachi H-7000). The monolayers were transferred onto a collodion-covered electron microscope grid at various surface pressures by a horizontal lifting or a vertical dipping method. Pt-carbon was vapor-deposited onto the monolayers for the observation of bright field image with a shadowing angle of 12°.

Measurement of Surface Pressure Anisotropy

Since the polypeptide monolayer is composed of rigid rod-like α-helix and the α-helix chains easily orient by the compression force, the anisotropy of surface pressure depending on the compression direction can be expected. Figure 1 shows the schematic representation of measurement of surface pressure anisotropy by using orthogonal Wilhelmy plates (15). The Wilhelmy plate was positioned in a parallel ($\pi_{//}$) or a perpendicular ($\pi_{\perp}$) direction to the compression direction. The Wilhelmy plate was fixed to prevent the free rotation of plate. If the monolayer is in a solid state, the surface pressure in the two directions ($\pi_{//}$, $\pi_{\perp}$) with respect to the Wilhelmy plate might be different, and if fluid-like structure, the magnitude of π and π should be the same.

Dynamic Viscoelastic Measurement

Dynamic viscoelasticity of bulk polypeptide films was measured at 11 Hz in order to investigate the temperature dependence of side chain motion. Rheovibron Dynamic Viscoelastomer DDV-II C (Orientec Co., Ltd.) was used for the measurement of temperature dependence of dynamic viscoelasticity. The bulk films of PMLG and PnHLG were cast from chloroform solutions.

Dynamic viscoelastic measurements of polypeptide monolayers at the air-water interface were carried out by applying the step-rise compression strain to the monolayers in a homogeneous state. A homogeneous state of the monolayer was determined by the observation of bright field image of monolayer. Surface pressure variation [π(t)] of the monolayers after the application of strain was measured by Langmuir balance (16). Dynamic storage modulus, E' and dynamic loss modulus, E" were evaluated by Fourier analysis of π(t) as shown in eq.(1).

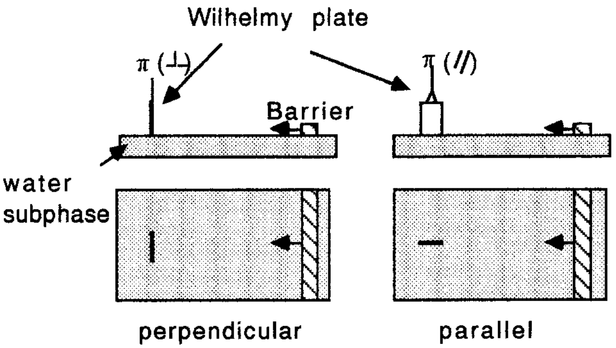

Figure.1 Schematic representation of the surface pressure anisotropy measurement. The Wilhelmy plate was suspended parallel ($\pi_{//}$) and perpendicular ($\pi_{\perp}$) to the compression direction of monolayer.

$$E^* = E' + i\,E'' = \frac{\pi_e}{\varepsilon} + 2\pi f\int_0^t \frac{\pi(t)}{\varepsilon}\sin(2\pi ft)dt + i2\pi f\int_0^t \frac{\pi(t)}{\varepsilon}\cos(2\pi ft)dt \qquad (1)$$

where π_e, ε and f are equilibrium surface pressure, strain and frequency, respectively.

Results and Discussion

Viscoelastic Properties of Polypeptide in a Bulk State

Figure 2 shows the temperature dependence of the dynamic storage modulus E', loss modulus E", and mechanical loss tangent, tanδ for the bulk films of PMLG and PnHLG. According to the previous NMR, dielectric, and mechanical measurements, six relaxation regions are discernible in the temperature range investigated (17). These are labeled α, β, γ, δ, ε, and ξ in order of decreasing temperature. The α-relaxation arises from the thermal molecular motion of the α-helices in the crystalline region and the β relaxation arises from micro-Brownian segmental motion of the disordered region or the disordered part of the α-helices. The γ and δ relaxation are associated with motions of the entire side-chain groups in the crystalline and the disordered regions, respectively. The molecular motions of the side chain groups occur without any change of the relative position along the α-helix axis between neighboring α-helix chains. The ε and ξ relaxations involve the conformational rearrangements of n-alkyl groups including carboxyl group and n-alkyl groups alone, respectively.

In the case of PnHLG, the δ absorption of E" was observed at around 220K, and the storage modulus E' decreased significantly at this temperature range. On the other hand, the δ absorption of E" was observed for PMLG around 250 K. However, the decrease of the corresponding E' was not

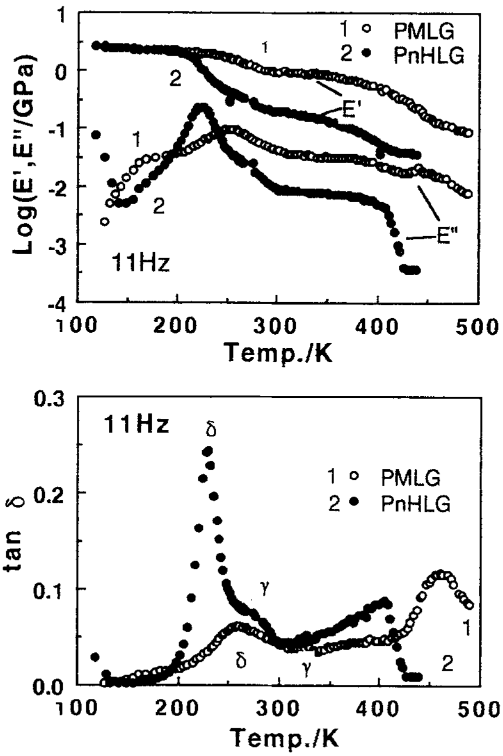

Figure 2. Temperature dependence of the storage modulus E', loss modulus, E" and loss tangent, tanδ for the bulk films of PMLG and PnHLG.

significant compared with that of PnHLG. This is ascribed that long side chain of PnHLG makes itself more plastic. The magnitude for E' of PnHLG is remarkably lower than that for PMLG owing to the active thermal molecular motion of long side chain at room temperature. This side chain motion should be reflected on the viscoelastic properties of monolayer at the air-water interface.

Surface Pressure (π) - Area (A) Isotherm and Aggregation State at Air-Water Interface

Figure 3 shows the surface pressure (π)-area (A) isotherm of PMLG monolayer and the corresponding bright field images at 18mN·m^{-1}, 20 mN·m^{-1} and 25mN·m^{-1}. A plateau was observed at surface pressure of around 20 mN·m^{-1}. Appearance of plateau region was attributed to a typical type of collapse for polypeptide monolayer : a transition from a monolayer to a bilayer (18,19). This can be confirmed from the bright filed image at 20 mN·m^{-1} showing linear humps oriented perpendicular to the compression direction. LB film was prepared for the evaluation of monolayer thickness at surface pressure of 18 mN·m^{-1} by a vertical dipping method. From the X-ray diffraction measurement of the PMLG LB film, the thickness of monolayer at 18 mN·m^{-1} was evaluated to be about 1 nm, which corresponded to the diameter of the α-helical rod-like molecular chain of PMLG. Also, infrared spectra of the PBLG film transferred to the ATR crystal showed characteristic absorption of α-helix. These results indicate that PMLG in the monolayer is in an α-helical conformation.

In the case of the PMLG monolayer compressed at 20 mN·m^{-1}, linear humps which oriented perpendicular to the compression direction were observed parallel each other. An appearance of linear humps indicates that the monolayer starts to collapse locally, even though the pressure is lower than the generally defined collapse pressure as the peak of π - A isotherm. The linear humps of PMLG monolayer appeared owing to its rod-like molecular structure. The number of humps increased with decreasing the surface area less than 0.1 nm^2·residue^{-1}. A surface pressure started to increase again at the surface area of ca. 0.1 nm^2·residue^{-1}. Finally, the monolayer was almost collapsed and three dimensional fibrillar structure was observed at 25 mN·m^{-1}. These morphological changes in a

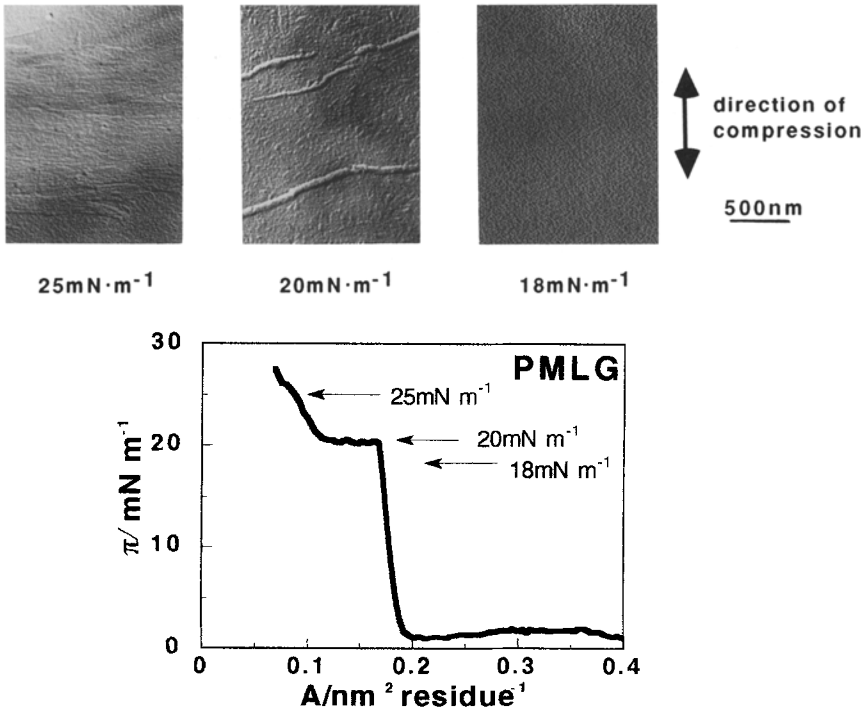

Figure 3. Surface pressure (π) - area (A) isotherm of PMLG monolayers and the corresponding bright field images at 18 mN·m^{-1}, 20mN·m^{-1} and 25mN·m^{-1}.

higher surface pressure region indicate that the α-helical chains in the PMLG monolayer are aligned parallel each other, lying their helical axes at the air-water interface. Also, these results indicate that inplane ordering of the polypeptide molecule can be achieved in the homogeneous state at 18 mN·m^{-1}.

Figure 4 shows the surface pressure (π) - area (A) isotherm of PnHLG monolayer and the corresponding bright field images at 15 mN·m^{-1} , 18 mN·m^{-1}, and 20 mN·m^{-1}. Aggregation process of PnHLG monolayer at the air-water interface was quite different to that of PMLG monolayer during compression, whereas a plateau was also observed at a surface pressure of 16 mN·m^{-1}. The bright field image of the PnHLG monolayer at 15 mN·m^{-1} exhibited a homogeneous state. The value of 15 mN·m^{-1} is below the surface pressure in a plateau region. Therefore, Figures 3 and 4 revealed that a homogeneous aggregation state of polypeptide monolayers was attained at a surface pressure just below a plateau region. The extrapolation of the first steep rise of the π -A isotherms to zero pressure gives an area per residue of 0.18 and 0.28 nm^2 for PMLG and PnHLG, respectively. This difference in area per residue is ascribed to the side chain length of PMLG and PnHLG molecule.

The linear humps for the case of PnHLG were not observed even at 18 mN·m^{-1} above the surface pressure in a plateau region, while the linear humps of PMLG were observed above that in a plateau region as shown in Figure 3 (b). At the surface pressure of 20mN·m^{-1}, disc-like collapsed region was observed for PnHLG monolayer. These difference in variation of aggregation structure of PMLG and PnHLG during a compression process can be explained based on the thermal molecular motion of a side chain group. As shown in the previous section, the magnitude of mechanical loss tangent, tanδ of side chain group for PMLG in bulk state was fairly lower than that of PnHLG. The magnitude of tanδ of side chain group for PMLG in the monolayer at the air - water interface was also expected to be lower than that of PnHLG, whereas the magnitude of tanδ at the air - water interface was not always the same magnitude as bulk state. The morphological observation in this study revealed that the thermal molecular motion of side chain group for PnHLG prevented the collapse of monolayer at the surface pressure above a plateau region.

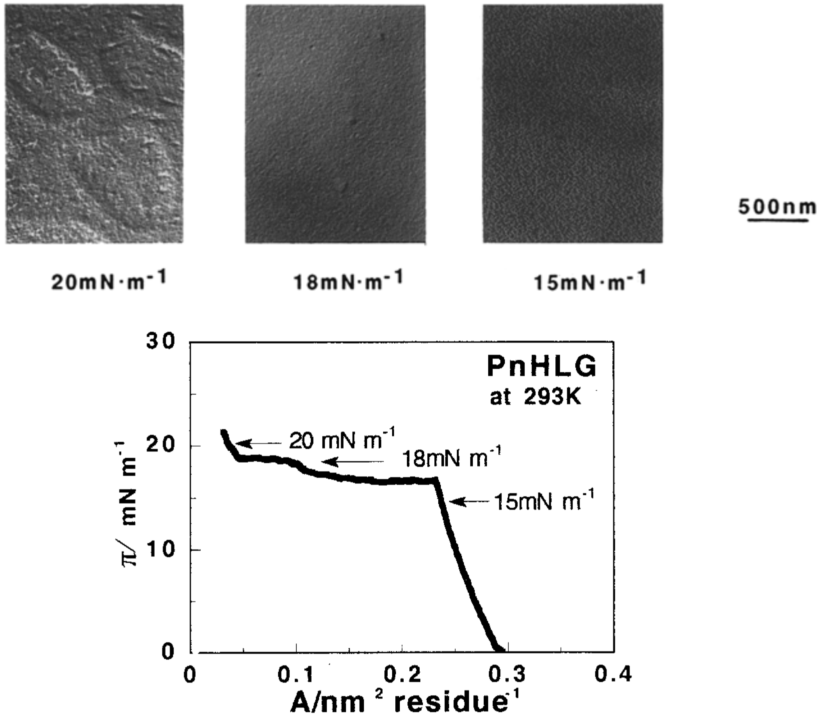

Figure 4. Surface pressure (π) - area (A) isotherm of PnHLG monolayers and the corresponding bright field images at 15 mN· m^{-1}, 18mN·m^{-1} and 20 mN·m^{-1}.

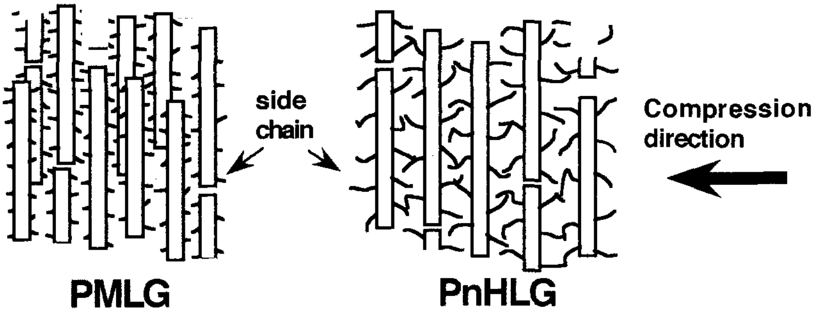

Figure 5. Schematic representation of aggregation structure of PnHLG and PMLG monolayers at the air-water interface with the surface pressure at plateau region.

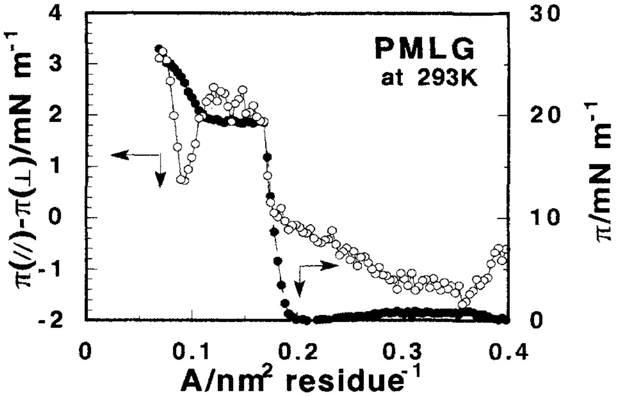

Figure 6. Surface pressure (π) - area (A) and difference between surface pressure recorded by Wilhelmy plate parallel to a compressional direction ($\pi_{//}$) and perpendicular to it ($\pi_{\perp}$) for PMLG monolayers.

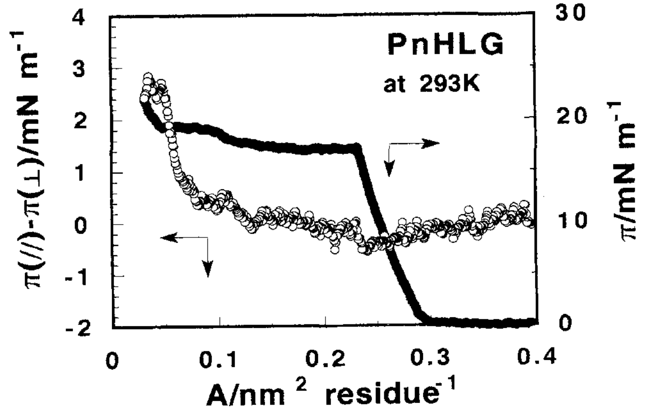

Figure 7. Surface pressure (π) - area (A) and difference between surface pressure recorded by Wilhelmy plate parallel to a compressional direction ($\pi_{//}$) and perpendicular to it ($\pi_{\perp}$) for PnHLG monolayers.

Figure 5 shows the schematic representation of aggregation structure for the PMLG and the PnHLG monolayers at the surface pressure of plateau region. Side chain of the PnHLG molecule in monolayer can act as viscous media among rigid rod like molecules owing to its active thermal molecular motion. On the other hand, the PMLG molecule which has non-active side chain motion aggregated as rigid rod like manner in the monolayer, resulting the collapse of monolayer at higher surface pressure. It is revealed that the aggregation structure of polypeptide monolayers is strongly influenced by their side chain motion.

Anisotropic Mechanical Properties of Monolayer

Figures 6 and 7 show the π- A isotherms and the difference between surface pressure recorded by orthogonal Wilhelmy plate parallel and perpendicular to a compressional direction ($\pi_{//}$and $\pi_{\perp}$) for PMLG and PnHLG monolayers, respectively. In the case of PMLG monolayer, the surface pressure difference between the parallel and perpendicular orientations, $\pi_{//}$ - $\pi_{\perp}$ was significant throughout a compression process. A large negative value of $\pi_{//}$- $\pi_{\perp}$was observed at the area larger than 0.2nm^{-1} . This might be due to the orientation of rigid rod-like PMLG molecular axis to the direction of compression. On the other hand,$\pi_{//}$- $\pi_{\perp}$ for PnHLG monolayers was almost negligible before reaching an end of plateau region. These results indicate that the surface pressure of PMLG monolayers is more anisotropic than that of PnHLG monolayer. Since the PMLG α-helix with a short side chain oriented perpendicular to the compression direction as concluded from TEM observation, the anisotropy of surface pressure increased with a decrease in occupied area. On the other hand, the α-helical PnHLG chain behaves like a rod like molecule dispersed in a viscous media since a long alkyl side chain has a side chain relaxation at lower temperature than subphase temperature. Then, the anisotropy of surface pressure can be easily released in a viscous side chain media. Thus, PnHLG behaves like an isotropic monolayer.

Recently, π - A isotherm of fatty acid monolayers at the air-water interface was investigated by using orthogonal Wilhelmy plate (15). Pentadecanoic acids (C_{15}) did not show a significant difference in surface pressures parallel and perpendicular at any point on a whole region of π - A isotherm, while stearic (C_{18}) and lignoceric (C_{24}) acids showed a difference in surface pressures parallel and perpendicular at all point. These results indicate that anisotropy of surface pressure well corresponds to the rigidity of monolayer.

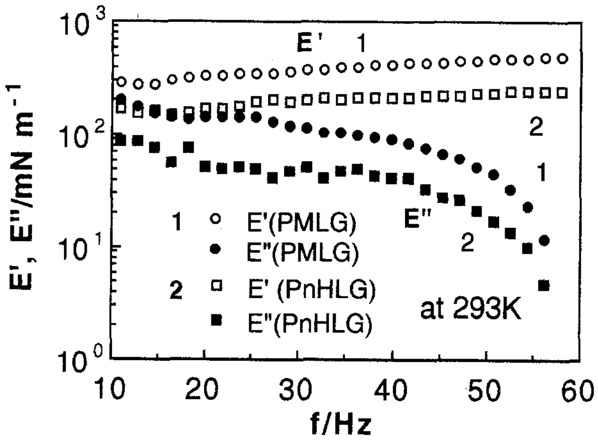

Figure 8. Frequency dependence of the storage modulus, E' and loss modulus, E" for PMLG and PnHLG monolayers at the air-water interface at 293K.

Dynamic Viscoelasticity of Monolayer at the Air-Water Interface

Figure 8 shows the frequency dependence of the storage modulus E' for PMLG and PnHLG monolayers at the air-water interface. E' of the PnHLG monolayer was observed lower than that of PMLG monolayer, due to a lower viscous media of the PnHLG side chain at 293 K compared that of PMLG one. These results indicate the viscoelastic properties of synthetic polypeptide monolayer are strongly depend on its side chain motions, because the thermal molecular motion in a side chain group is more directly enhanced owing to a side-by-side connection of the α-helical cores in the two dimensional polypeptide monolayer.

The storage modulus E' per unit area was evaluated from the storage modulus E' per unit length given in Figure 8. The thickness of PMLG monolayer was evaluated from the X-ray diffraction measurement of the LB film. Then, the storage modulus E' per unit area was evaluated about 0.3 ~ 0.5 GPa for the PMLG monolayers at the air-water interface. This calculated storage modulus E' corresponds to the modulus perpendicular to molecular axis. On the other hand, bulk modulus E' of oriented poly(γ-methyl-D-glutamate)(PMDG) film perpendicular to the orientation direction has been revealed to be ca. 0.7 GPa at room temperature in our laboratory. This bulk modulus E' of polypeptides was measured for the perpendicular direction of molecular axis in oriented film. The monolayer's modulus E' perpendicular to molecular axis at the air - water interface is clarified almost the same as the bulk modulus E' for the perpendicular direction of molecular axis in oriented film. In the case of polypeptide, intermolecular interaction is very weak even bulk state, because of the formation of the helix by the intramolecular hydrogen bonding. The weak intermolecular interaction between α-helix makes modulus of bulk state and monolayer at the air-water interface almost the same magnitude.

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